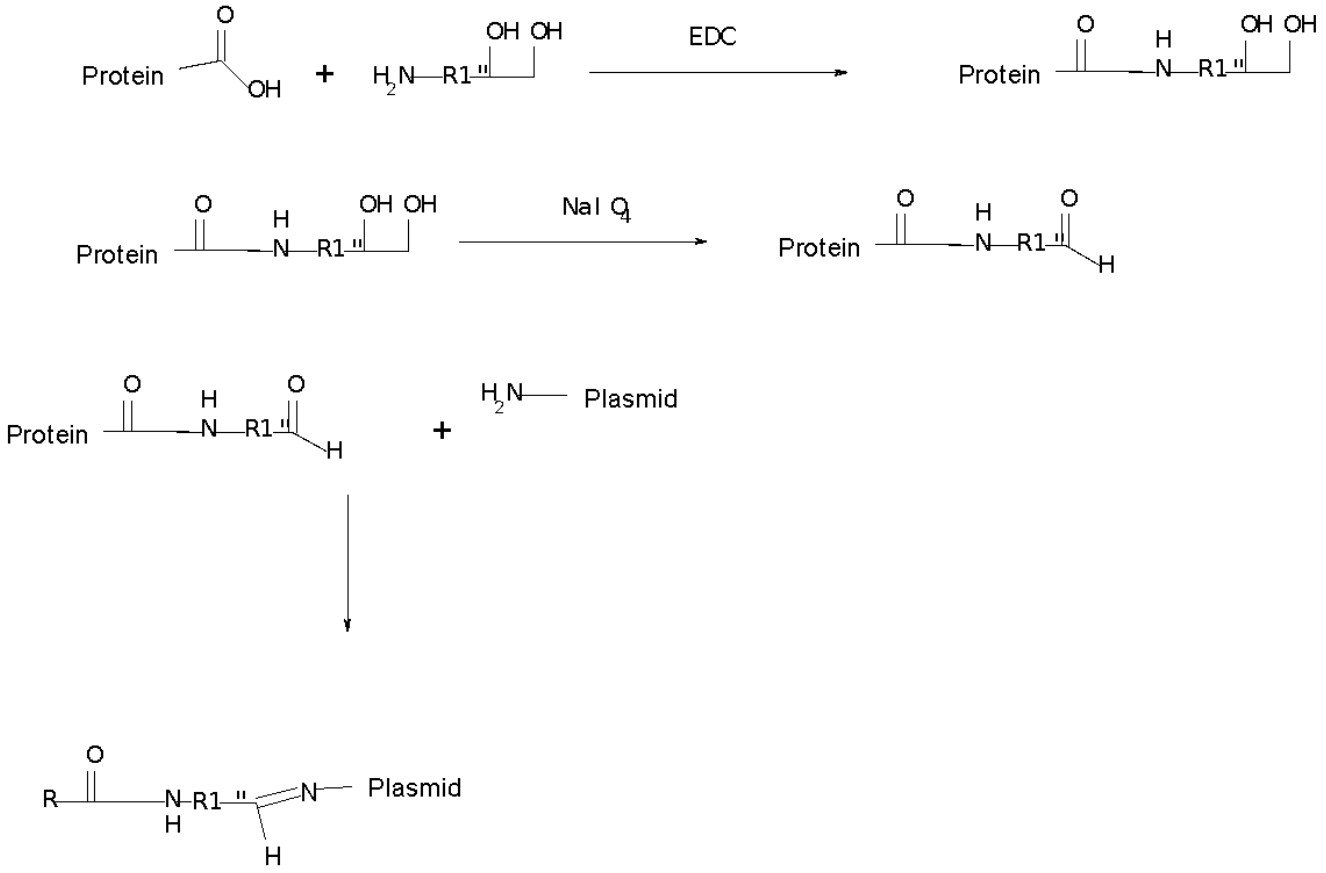

Materials Required

- Carrier protein: 2mg cholera toxin
- Conjugation Buffer: 0.1M MES (2-[*N*-morpholino]ethane sulfonic acid), pH 4.5-5 (Product No. 28390)
- EDC: 10mg
- 3-amino-1,2-propanediol : 1-2mg
- Oxidation Buffer: 0.1M sodium acetate buffer, pH 5.5. (As described in the Important Product Information, phosphate-buffered saline, pH 7.2 may be used as an alternative but is not as efficient for periodate oxidization.)

• Phosphate-buffered saline (PBS; e.g., 100mM sodium phosphate, 150mM sodium chloride; pH 7.2; Product No. 28372)

• Sodium *meta*-periodate (Product No. 20504)

• 1mL of 1N sodium hydroxide (NaOH) to make AminoLink Reductant stock solution

• Blocking Buffer: 1.0M Tris•HCl, pH 7.4 or 1.0M ethanolamine, pH 9.6

• cut-off membrane 3000 Dalton.

Procedure

1- Binding 3-amino-1,2-propanediol to protein

1. Equilibrate EDC to room temperature.
2. Add 2mg of lyophilized cholera toxin to 200µL Conjugation Buffer.
3. Dissolve up to 2mg of the 3-amino-1,2-propanediol in 500µL of Conjugation Buffer and add it to the 200µL carrier protein solution.
4. dissolve 10mg of EDC in 1mL of ultrapure water and immediately add 100µL of this solution to the carrier-peptide solution.
5. React for 2 hours at room temperature.
6. Purify the conjugate using a cut-off membrane of 3000 Dalton. And an Oxidation Buffer: 0.1M sodium acetate buffer, pH 5.5.

2. Oxidation with $NaIO_4$ and amino-reduction with plasmid

1. Dissolve 0.5-10mg of protein in 1mL of Oxidation Buffer.
2. Prepare 20mM periodate solution by dissolving 4.3mg of sodium *meta*-periodate per milliliter of Oxidation Buffer. Keep solution on ice and protect it from light.
3. Add 1mL of cold sodium *meta*-periodate solution to 1mL of the protein solution and mix well. Allow the oxidation reaction to proceed in the dark for 30 minutes on ice or at 4°C.

4. Dialyze samples overnight against PBS, or use a desalting column equilibrated with PBS to remove excess periodate and exchange the buffer .

5. Dissolve an amine- or hydrazide-containing molecule (e.g., plasmid) at a concentration of 10mg/mL in PBS.

6. Mix the oxidized and desalted protein solution with the plasmid solution in amounts necessary to obtain the desired molar ratio for conjugation. Often, the second molecule is reacted in 4- to 15-fold molar excess over the amount of oxidized protein.

7. Add 10µL of 1M NaOH Solution.

8- Allow reaction to proceed for 4-6 hours at room temperature.

9- Block non-reacted aldehyde sites (quench the reaction) by adding of 50µL of Blocking Buffer per milliliter of conjugation solution and incubating the reaction for 30 minutes at room temperature.

10- Purify the conjugate from excess reactants using a desalting column (as described in Section B) or dialysis (see Related Thermo Scientific Products). Exchange the conjugate into a storage buffer (PBS) .

*To reduce the imine bond replace the solution at step (2.7) by 10µL of 160mg of AminoLink Reductant ($NaCNBH_3$) in 0.5mL of 1M NaOH (results in 5M stock).